

# When Asthma Strikes



Pollen has long been believed to cause asthma, a disease that affects hundreds of millions worldwide. But it's also known that pollen grains are too big to get deep into the lungs, where asthma strikes. So how does pollen trigger asthma? Now researchers think they know the answer.

More than 14 years ago, on a summer night, thunderstorms swept through England, and from the rain and lightning emerged one of the largest asthma epidemics in history. Up to 10 times more asthmatics flooded emergency rooms than normal. In the North Thames region, over a thousand people went to the hospital complaining of asthma attacks. In other areas, several hundred checked into the emergency rooms. Many of those people had never had asthma before. Most people don't think about epidemics as occurring in one evening, but this one hit during the night of

June 24, 1994. A huge number of people suffered from a very dangerous disease over a very short period of time.

The cause of this countrywide asthma attack was linked to pollen, which the American Lung Association lists as one of the triggers of asthma. Although pollen has long been considered a cause of asthma, the exact mechanism is unknown. For decades, scientists have known that pollen grains are too big to penetrate deep into the respiratory tract, meaning pollen shouldn't be able to trigger asthma. It's been a real puzzle.

The correlation between pollen and asthma, as measured by the medical community, has been only qualitative. As of now, we think we've got a pretty good hypothesis. But ultimately, we'd like to find an even more detailed and quantitative explanation.

At the same time, we're working on ways to minimize the prevalence of asthma attacks by providing real-time warnings. The World Health Organization estimates that asthma affects 300 million people. In 2005, asthma killed 255,000 people. I may not be able to cure the disease, but I'd like to be able to tell parents, school teachers, and school principals when it might be a good idea for asthmatic children to stay indoors.

Asthma is a disease of the respiratory tract, which starts with the nasal passage and the trachea, or windpipe. The trachea eventually splits into two tubes called bronchi that go into each lung. The bronchial tubes in turn go through about 18 different generations of branching inside your lungs,

Don't sneeze!  
The morning wind  
stirs up ryegrass  
pollen near Eugene,  
Oregon.

By Richard C. Flagan

each time shrinking in diameter. The branching ultimately leads to the bronchioles, which connect to the alveoli, the structures where gas is exchanged. The alveoli, which look like a bunch of grapes, have lots of surface area to absorb oxygen—about 70 square meters, bigger than some New York City apartments. In the upper portion of the respiratory tract, the tubes are lined with cartilage rings that provide structural support. But by the time you get past somewhere between the fifth and eighth branching, that support diminishes dramatically, and this is where asthma strikes.

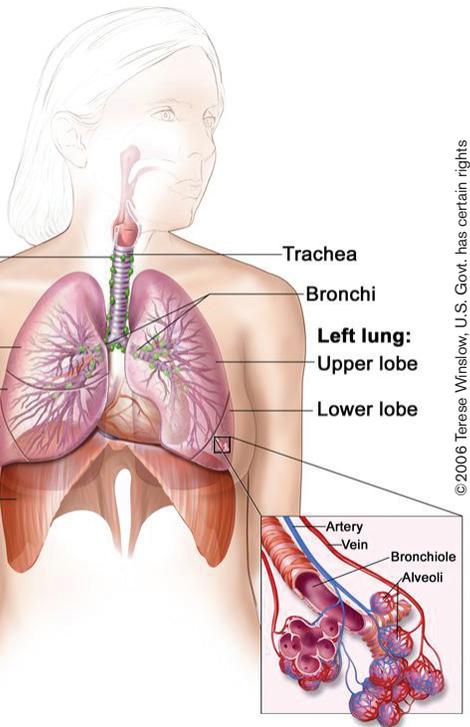
When you inhale, you flex your diaphragm to expand your bronchial tubes, bronchioles, and lungs, increasing their volume and lowering their internal air pressure to below that of the outside air. Since air always flows from high to low pressure, air is drawn into your lungs. When you exhale, your muscles relax, and your respiratory system gently compresses, decreasing its volume and increasing the air pressure so air flows out. An asthma attack happens when the bronchial tubes and the bronchioles—which are normally up to three millimeters in diameter—constrict due to some kind of irritation. Problems arise during exhalation, when the bronchioles are already narrowing. Further constriction from asthma makes it harder for air to flow out, and if it's hard for air to flow out, there's no room for fresh air. That makes it difficult to breathe, and you often hear wheezing from asthma sufferers. The lung also reacts to irritation by pushing air out, which constricts the bronchioles even more.

The bronchioles could collapse, and that's when asthma becomes life threatening.

My lab has been studying pollen in the Willamette Valley, near Eugene, Oregon. The area has many square miles of commercially grown ryegrass, which makes for longer-lasting green lawns and produces one of the most allergenic pollens associated with asthma. The picture on the opposite page shows the morning wind spreading the pollen. It looks like fog, but it's not—it's pollen. It makes me sneeze just looking at it.

In the summers of 2005 and 2006, we worked with Kraig Jacobson, an allergist in Eugene. We collected and analyzed pollen samples, measured the allergens, and looked at data from local clinics and hospitals. The data shows a strong visual correlation between the amount of respiratory allergens and the number of emergency room visits, but we need a lot more data for any clear, statistically confident conclusions.

Every flowering plant species makes pollen grains of unique shapes and sizes. People measure pollen by counting grains, a very tedious exercise in which people stare through a microscope to identify them. Pollen grains range from about 20 to 100



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microns in diameter (a micron is a millionth of a meter).

Some plants depend on insects or birds to pollinate, producing sticky pollen to better attach to the pollinating animal. The pollens that we're studying aren't sticky, but have evolved to disperse in a gentle wind. Photographs of pollination often show a cloud of pollen dispersing from the plant, but in nature, pollen doesn't become airborne spontaneously—only when it's disturbed. In most pictures of that sort, someone's hand is actually shaking the branch.

A ryegrass flower opens up during the day when the sun dries the air. The outer layer dries and tears open, exposing the pollen grains inside.



After the pollen is released, wind carries it to a random landing spot. If it happens to land on a female flower, then there's an exchange of genetic material to fertilize the seed. Once the pollen lands on the flower, something called a tubule grows out of the aperture, which is a small opening on the pollen grain's tough outer wall. An extension of the cell wall, the tubule stretches out, seeking out the flower's embryo sac, which will eventually become a seed. Ryegrass pollen tubules can extend tens of times the diameter of the original pollen grain in a matter of hours—it's really an amazing process.

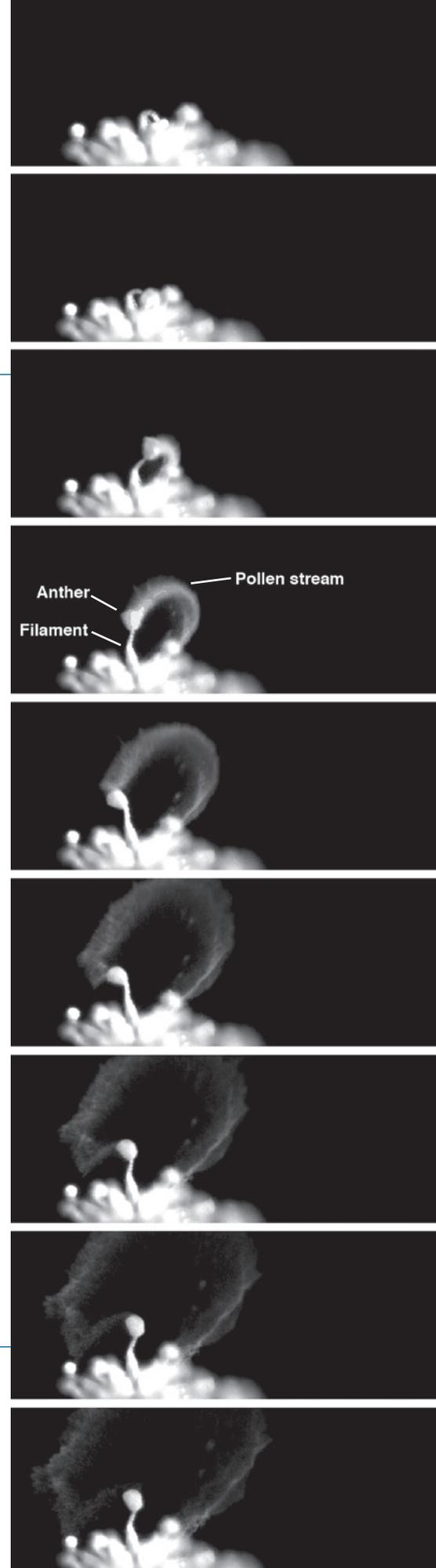
Pollen is held in the male flower's anthers. When the anthers, the pollen is released. Much of the classical botany literature anthropomorphizes what happens, saying that the anthers "disperse" the pollen upon opening, as though there's an active compulsion on the part of the plant to do so. But usually there's no propulsive motion—when you disturb many plants, the pollen falls straight down or gets carried away with the wind. When a ryegrass flower opens in still air, most of the pollen just sits there. In the summer of 2004, Grace Lu, a high-school student from Kennesaw, Georgia, worked in my laboratory as part of the Research Science Institute, a national program that puts high-school students in college labs. She took some absolutely fantastic pictures that show the three-millimeter-long ryegrass flower exposing its pollen to the environment. The flower, which looks like a little pea pod, opens up during the day when the sun lowers the humidity. The outer layers dry out,

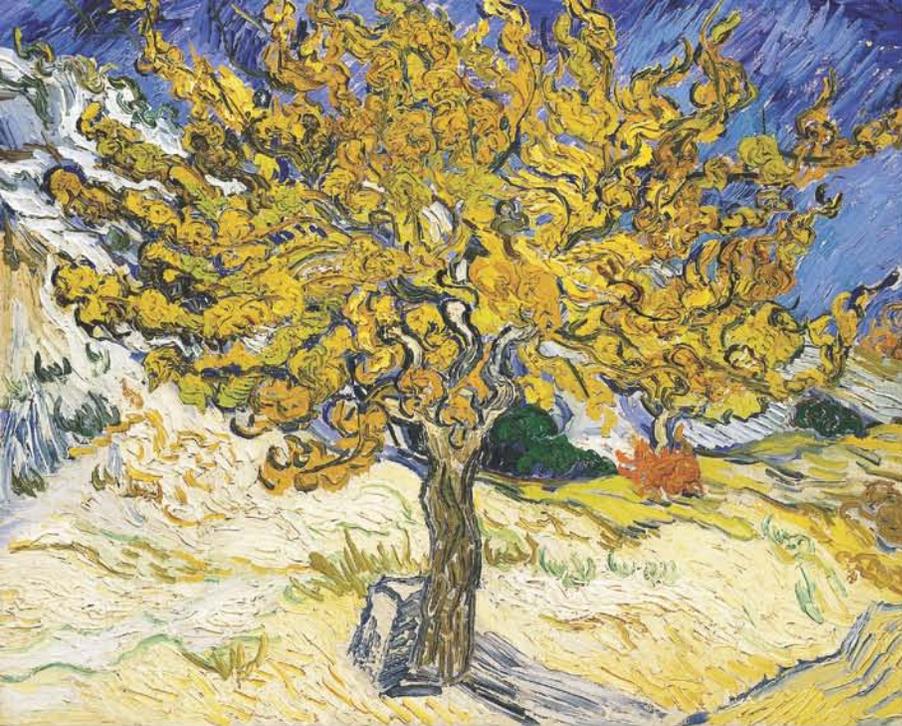
shrink, and tear open, but when the humidity rises at night, the outside soaks up some water and partially closes back up. The intermolecular forces between the pollen and the surface of the anther, called van der Waals forces, are powerful enough to hold the pollen in place until something causes it to fall down. But releasing pollen in this way is not always the case, as you shall see.

#### FASTER THAN A SPEEDING POLLEN GRAIN

While looking at the wind speeds required to pull stray pollen off plants, we made an amazing observation. One day in March 2005, Boswell Research Fellow Phil Taylor, a botanist in my lab at the time, brought in some flowers from a mulberry tree. Mulberry flowers are only a few millimeters in size, and they cluster around individual stems. He put one of these stems in a test tube and got ready to run the experiment. But when he looked back, he saw a halo of pollen around the flower on the tabletop as far as 10 centimeters from the plant. How did the pollen get so far? I did a back-of-the-envelope calculation and found that the pollen had to leave the flower at a velocity of about 200 meters per second. Now, the speed of sound is roughly 340 meters per second, so the pollen was traveling incredibly fast. Naturally, we had to ask how this works.

We didn't have any instruments fast enough to capture this phenomenon, so I enlisted the help of Michael Dickinson, the Zarem Professor of Bioengineering,





Left: Van Gogh's *Mulberry Tree*, October 1889, Norton Simon Art Foundation, gift of Mr. Norton Simon. Far left: These time-lapse images capture the mulberry tree slinging its pollen into the air. The time between each frame is 1/2,000th of a second.

who uses high-speed cameras that shoot 10,000 frames per second to study insect flight. Along with a graduate student in his lab, Gwyneth Card, we filmed the mulberry plant releasing pollen. We didn't get good pictures until late in the season, when the plants were weak and couldn't expel the pollen with much vigor. At the peak of the season, we couldn't catch it with a 10,000-frame-per-second camera—the movement was too fast. We'd record one frame in which nothing's happening, and in the next, there's a streak showing the pollen's path. By measuring that distance, and assuming it was covered in 1/10,000th of a second or less, we calculated that the pollen was shot out at speeds as high as 237 meters per second. That's 530 miles per hour, or Mach 0.7. We took several measurements, and found speeds ranging from a few tens of meters per second to a couple hundred. Our film is the fastest recorded motion of any living thing on this planet—and it's a tree. At its peak, mulberry pollen can beat a cheetah in a race, albeit only for 10 centimeters.

Now when you discover something like this, you want to find out if anyone else has ever seen it before. We dug through the

literature and found an early reference from Pliny the Elder, a Roman naturalist from the first century AD. He states, "The germination, when it has begun, bursts forth all over the tree at the very same moment; so much so, indeed, that it is accomplished in a single night, and even with a noise that may be audibly heard," [Pliny the Elder, *The Natural History, Book XVI, The Natural History of the Forest Trees*, eds. John Bostock and H. T. Riley]. The editors footnoted the noise as "a mere fable, of course." But I've heard that noise. It's real. An orchard of mulberry trees might very well produce a rustle. This story reminds us that we always want to read the literature. It's very dangerous to think that we're the first to see (or hear) something.

The high-speed camera showed that the mulberry flower releases its pollen like a catapult. The catapult in this case is the stamen, the flower's male organ. It's composed of a stalk, called the filament, and at its tip is the anther, which holds the pollen. In mulberry flowers, stamens are grouped into fours, and each filament is bent over. At night, the flower absorbs water from the air, which increases the pressure inside the filament, causing it to begin straightening and pressing against another part of the flower

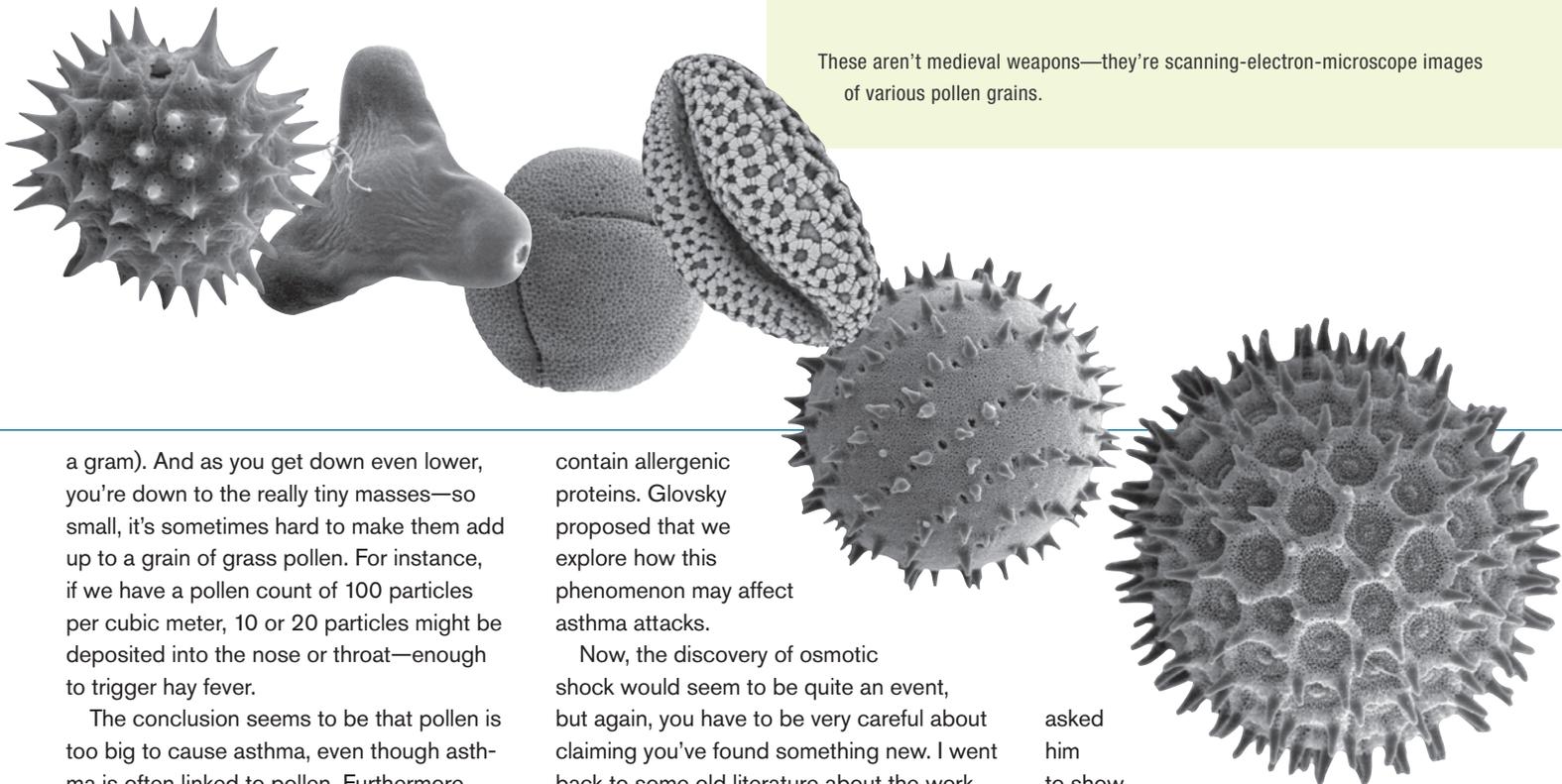
called the pistillode. Tension builds. The low humidity of the next day's warm air leads to a slight movement of the filament as the exposed parts dry. This causes the fine hairs on the anther to tear, which helps the anther open and expose the pollen. The filament ultimately slips past the pistillode, releasing the built-up elastic energy and flinging the pollen out at high speeds. The entire straightening of the filament takes less than 25 microseconds.

#### **BIG POLLEN, TINY BRONCHI**

Let's step back for a moment and look at what happens when a particle enters our respiratory system. Where particles get deposited is a function of their size. Big particles—those larger than 20 to 30 microns in diameter—have too much inertia to be sucked deep into the lungs, and instead deposit in the upper respiratory tract, which goes from the nose to the throat. But as the particles get smaller, the efficiency with which they are deposited drops off dramatically and they penetrate deeper, finding their way into the lower respiratory tract, which starts from the bronchi on down. The smallest particles make their way to the lower airways of the lung—deep in the bronchial region, where they may deposit, leading to an inflammatory response that induces bronchial constriction. The particles associated with respiratory irritations, such as cigarette smoke, viruses, and diesel soot, are all very small, and this is where they wind up. But pollen is huge, and although it covers a wide range of sizes, it's too big to get into the lower regions of the respiratory system.

A five-year-old child running around the park, for example, might get 10 to 20 nanograms (billionths of a gram) per hour of material in the throat and the first few branches of the bronchial tubes. Deeper in the bronchial region, the total mass drops down to about 100 picograms (trillionths of

Our film is the fastest recorded motion of any living thing on this planet—and it's a tree.



These aren't medieval weapons—they're scanning-electron-microscope images of various pollen grains.

a gram). And as you get down even lower, you're down to the really tiny masses—so small, it's sometimes hard to make them add up to a grain of grass pollen. For instance, if we have a pollen count of 100 particles per cubic meter, 10 or 20 particles might be deposited into the nose or throat—enough to trigger hay fever.

The conclusion seems to be that pollen is too big to cause asthma, even though asthma is often linked to pollen. Furthermore, asthma incidence has doubled throughout the world despite improvements in air quality. Asthma epidemics also accompany thunderstorms, and in many cases patients show high sensitivity to grass pollen. The mystery, then, is how pollen causes asthma.

In 2000, before he came to Caltech, Phil Taylor and Bruce Knox at the University of Melbourne published a paper that caught the eye of Michael Glovsky, a Pasadena allergist and immunologist, who is now also a visiting associate in chemical engineering at Caltech. The paper described what happens when you put pollen in water. The pollen undergoes something called osmotic shock, causing it to rupture. Osmosis is when water diffuses across a membrane separating, say, a low-salt concentration from a high-salt concentration. In a high-humidity environment, water diffuses into the pollen grains, increasing the pressure until they burst like overfilled water balloons. The cytoplasmic material inside the pollen spills out into the water, producing small fragments that include bits of organelles and hundreds of starch granules, some of which

contain allergenic proteins. Glovsky proposed that we explore how this phenomenon may affect asthma attacks.

Now, the discovery of osmotic shock would seem to be quite an event, but again, you have to be very careful about claiming you've found something new. I went back to some old literature about the work of Robert Brown, the Scottish physician-turned-botanist who observed Brownian motion back in 1827. Brownian motion is the random movement of small particles suspended in a liquid or gas. This movement is a key piece of evidence for the existence of atoms and molecules, which bump into the particles and cause them to move. Many people think that Brown did his experiment with pollen, but the grains are too big for Brownian motion. In fact, he did it with small particles within the pollen grain and with those fragments that come out of the pollen, so he had observed rupturing pollen more than 150 years ago.

We invited Taylor, who had just earned his PhD in Melbourne, to come to the lab that summer. For his doctoral thesis, he had proposed a mechanism for how pollen is dispersed into the air. He speculated that once shed from the plant, the pollen lands on leaves, the grass, and the ground. The pollen then gets wet from rain, morning dew, or high humidity, and ruptures. After the water eventually dries up, a breeze blows the pollen fragments away. When he arrived, I

asked him to show me how his hypothesis worked. I

had been working on a new method for detecting explosives in luggage by looking for chemical traces on the outside of the bag. I had looked at how air could blow particles off a surface into my sampler, so I was familiar with the underlying physics of this scenario. Taylor's idea was plausible, but I didn't think the wind would be strong enough to carry away the pollen fragments. We made a small wager: I told him to put the particles on a small, glass slide, let them dry, and then try with any air supply in our lab to blow the pollen off. He couldn't do it, even with 100 pounds per square inch of pressure, and I got a very nice bottle of Australian wine. So a month after he defended his thesis, Taylor disproved its main hypothesis.

Still, we thought the essence of his argument about the wet and dry cycle was correct. Every day, he would go out and gather pollen from different plants around campus and bring it back to the lab for analysis. Then, one day, he came in complaining that his feet were wet from walking through the

morning dew. This got him thinking about what happens to a flower when it gets wet, so we started studying flowers. We put ryegrass flowers in a glass box and lowered the humidity inside, simulating the dry sun during the day. The flowers' outer layers dried and opened. The flowers closed again when we raised the humidity to simulate nighttime conditions. Then, to explicitly see the effect of water, we sprayed mist on the flowers. When we lowered the humidity again, the flowers opened and exposed both pollen grains and fragments. In previous experiments, when we didn't spray water mist, which ensures pollen rupture, we just got big particles. So we learned that water, or humidity sufficiently high to rupture pollen grains, was crucial to producing asthma-causing particles.

Taylor ended up staying at my lab as a postdoc, and we continued working on the problem, studying rupturing pollen grains and looking for ways to blow pollen off surfaces. For example, we calculated how strong the wind must be to sweep pollen away. The surfaces of a flower's anthers and pollen grains are polymers of known composition. We approximated the pollen as a small polymer sphere and estimated the

strength of the van der Waals forces needed to bind it to the surface. From that, we could calculate the necessary wind speeds, which depend on the size of the particle. We found that dislodging a typical pollen grain would require a wind blowing at over 100 meters per second—that's greater than hurricane speed. This result, of course, isn't very realistic, since plants still pollinate, people still get hay fever, and asthma epidemics still hit when there are no hurricanes.

we still need to measure allergens with a higher time resolution, to get a more quantitative, detailed understanding of how pollen causes asthma, we have a plausible mechanism for how this process works, and it works for most plants. After the pollen ruptures because of the presence of water—high humidity, rain, or morning dew, for example—the fragments are trapped inside water droplets, which bead up due to the hydrophobic surfaces in the flower.

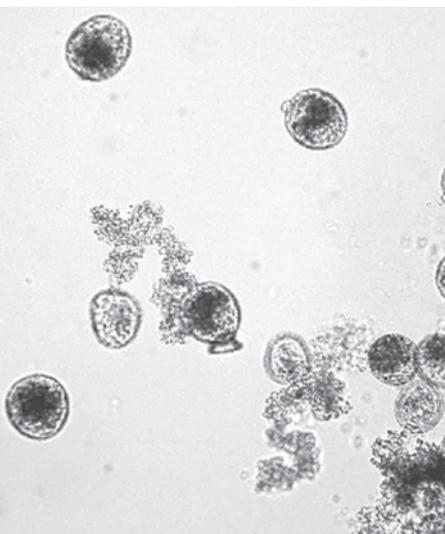
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It turns out that for Taylor's original idea to work, the pollen has to rupture in the special environment provided by the anthers in the flower. An anther's surface isn't smooth, but has many tiny bumps that make a textured surface. The surface of the pollen grain itself often has texture as well, and the tiny bumps reduce the contact area between the grains and the anther, minimizing the van der Waals forces between the two. The tiny bumps result in very low adhesion (and a hydrophobic surface—that is, one that's water-repelling), allowing the pollen to be swept up more easily than the initial wind-speed calculations had suggested.

Now we have the smoking gun. Although

Once the water dries, wind disperses the fragments, which are small enough to reach the bronchioles in the lung.

Since plants only disperse their pollen once a year, we have to take our measurements on whatever plants happen to be pollinating at the time. Fortunately, at least for us, one prolific pollinator is a tree found on the Caltech campus—the Chinese elm, which produces highly allergenic pollen. In 2004, we monitored airborne Chinese-elm pollen levels during its mid-August to mid-September pollination season. A staff scientist, Ann Miguel, took data from the roof of Keck Laboratory using a device called an impinger, a glass tube filled



Pollen grains rupture via osmotic shock. Water diffuses into the grains, causing them to burst like overfilled water balloons. This phenomenon disperses pollen fragments, which may contain asthma-causing allergens.

Right: Caltech's pollen group in front of a row of Chinese elm trees on campus. Naturally, these highly allergenic trees line the athletic fields and tennis courts. From left to right are Michael Glovsky; James House, visiting associate in chemical engineering; and Richard Flagan.  
Far right: Chinese elm tree flowers.



with water. As air is bubbled through the tube, the pollen collects in the water. We measured the number of pollen grains and their distribution, and found that there were huge numbers of smaller particles that can penetrate far down into the bronchioles. At the same time, we measured temperature, humidity, and wind speeds to see if we could find any correlations. We saw high pollen counts until the Santa Ana winds—hot, dry winds that sweep through Southern California every summer—came, killed off the flowers, and blew away the pollen. Two weeks after the Santa Anas, we still saw small peaks of allergen-containing pollen fragments, even though trees had long since stopped releasing pollen into the air. These spikes occurred about two days after nighttime humidity rose above 80 percent. So even though there was minimal pollen in the air, there were still asthma-causing pollen fragments, implying that pollen counts aren't good warnings for asthma epidemics.

We're also looking at how thunderstorms—the catalyst for that epidemic in England—fit into the picture. We don't know the details of how thunderstorms cause asthma epidemics, but we have a plausible idea. Dry updrafts blow pollen grains into the clouds, where high humidity causes them to rupture. Downdrafts and outflows then bring the pollen fragments back toward the ground, increasing the asthma risk. Thunderstorms also build electric charges, and positive ions from the ground can attach themselves to pollen. The electric charges may help the pollen rupture, but no one's

sure yet. Unfortunately, Southern California is not the best place to study this problem, since we only get a few thunderstorms each year.

#### SAVING LIVES

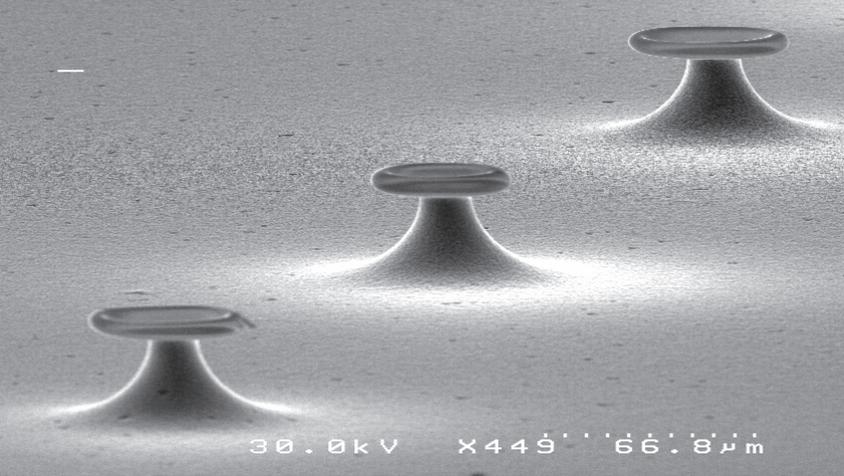
Now that we've looked at how pollen is dispersed, we can think about how to measure its risk. The pollen count is an after-the-fact measurement that tells people when to worry about hay fever. But by the time enough data has been taken to produce accurate pollen counts, people are already sneezing. And while hay fever is a huge problem, it's not a life-threatening disease like asthma.

Counting pollen isn't a good measurement for gauging asthma risk because it doesn't attack the right range of particles. We need to measure the respirable allergen, the pollen fragments that are capable of depositing at a rate of nanograms per hour in the bronchioles. These allergens vary dramatically from day to day—and probably even hour to hour—so we need a highly sensitive, fast-responding airborne allergen detector. We're working to adopt antibody

assays that people use in biochemistry laboratories all the time.

To do an antibody assay, you adhere an antibody to a surface. You put in your antigen, the allergen in this case, which binds very selectively to the antibody. Then you bring in another antibody that's labeled with fluorescent dye. This second antibody binds to the allergen-antibody couplet, and the whole thing lights up when under ultraviolet light, betraying the allergen. But this technique requires a lot of steps, two expensive antibodies, and a lot of time. It's not practical for making real-time measurements.

With Andrea Armani, an assistant professor at USC and a former graduate student [PhD '07] in the lab of Kerry Vahala, the Jenkins Professor of Information Science and Technology and professor of applied physics, we're developing a device that just may help us give real-time asthma warnings. This new tool is several orders of magnitude more sensitive than conventional assay techniques. The device, called an optical resonator, depends on a phenomenon known as the whispering-gallery mode. You may have experienced this when you go inside a rotunda and stand at one end of the



A scanning-electron-microscope image of the resonator. Light zips around the donut-shaped structure at its resonant frequency. When an allergen binds to an antibody attached to the ring, the resonant frequency changes according to the number of allergen molecules binding, allowing researchers to measure its concentration.

dome. You can speak in a whisper and the sound will bounce along the circular wall to the far end, where someone else can hear you. The resonator is a miniature version of the rotunda and does exactly the same thing, but with light. We shine light from an optical fiber into a little donut-shaped piece of silicon sitting atop a sloped pedestal. The ring is about 80 microns in diameter—about the width of a human hair—and seven microns thick. The light races around the perimeter at a resonant frequency that depends on the size of the ring. If we put an antibody that binds to our allergen on the ring, and pump in liquid containing the allergen, it binds to the antibody, and its presence shifts the resonant frequency of the light. By measuring the shift, we can detect single antigen-molecule binding events.

To make the resonators, we start with a block of silicon that has a two-micron layer of silica (also known as silicon dioxide, the chief ingredient of glass) on its surface. We use lithography techniques—the same techniques that are used in making micro-electronic devices—to turn the silica layer into an array of glass disks on the surface of the silicon. We then etch away the exposed silicon between the disks to make an array of glass-topped pillars. The underlying silicon etches faster than the glass on top, so the etching process undercuts the glass disks to make pedestals. Then we zap each disk with a laser, which melts the perimeter of the glass disks, causing it to bead up and thicken into a donut. Next, we couple each disk to an optical fiber and immerse

the array in a liquid under a cover slip. We can inject microliters of liquid containing the material we want to analyze into this microaquarium.

Right now, the device is still on the optical bench, hooked up to expensive machines. While we're using the resonator to measure biological molecules in the air, others may use it to solve other problems in biology or environmental science. For example, Rosen Professor of Biology and Professor of Bioengineering Scott Fraser is using it to learn about embryo development. In terms of measuring allergens, we're still far away from making a portable instrument, but it has the potential to help prevent not only asthma attacks, but also asthma-related deaths. And that's nothing to sneeze at. **ESS**

*Richard C. Flagan is executive officer for chemical engineering, the McCollum-Corcoran Professor of Chemical Engineering and Professor of Environmental Science and Engineering. Before joining the Caltech faculty in 1975, he earned his BSE (1969) in mechanical engineering at the University of Michigan, and an SM (1971) and PhD (1973) from the Massachusetts Institute of Technology. Among his many awards and honors are the American Chemical Society's Award for Creative Advances in Environmental Science and Technology in 2007, the Fuchs Memorial Award from the American Association for Aerosol Research in 2006, the Thomas Baron Award in Fluid-Particle Systems from the American Institute of Chemical Engineers in 1997, and the David Sinclair Award of the American Association for Aerosol Research in 1993. In 2004, Lund University in Sweden awarded him an honorary doctorate of technology.*

*This article was adapted from his May 2008 Seminar Day talk by Marcus Y. Woo.*